Oligonucleotide Properties Calculator

Enter Oligonucleotide Sequence Below
OD and Molecular Weight calculations are for single-stranded DNA

Nucleotide base codes

ATT GAA ACA CAA AAT ACC AGT TCT CAA ATA CAA TGA ACA TTA TTA ATT ATA ATT CAG TTA
AAA GTC ATT GAT CAG AAC AGC AGT GAA GGT TAG CTA TAA GCG CGT TAT AGG TGC AGG CAG
AGT GTC GTG CCT ATA TAT ACC CTT TGG AAT GCA CAA GTT GAA CAC AAA GAA AAA TG

Reverse Complement Strand(5' to 3') is:

CAT TTT TCT TTG TGT TCA ACT TGT GCA TTC CAA AGG GTA TAT ATA GGC ACG ACA CTC TGC
CTG CAC CTA TAA CGC GCT TAT AGC TAA CCT TCA CTG CTG TTC TGA TCA ATG ACT TTT AAC
TGA ATT ATA ATT AAT AAT GTT CAT TGT ATT TGA GAA CTG GTA TTT TGT GTT TCA AT

Number of Fluorescent tags per strand:

0 6-FAM 0 TET 0 HEX 0 TAMRA

Minimum base pairs required for single primer self-dimerization: 5

Minimum base pairs required for a hairpin: 4

Calculate | SWAP STRANDS | BLAST2 | Check Self-Complementarity

Physical Constants	Melting Temperature (T_M) Calculations
Length: 176 bases	1 75 °C (Basic)
GC content: 35 %	2 89 °C (Salt Adjusted)
Molecular Weight: 54503.7	3 79 °C (Nearest Neighbor)
1 ml of a sol'n with an Absorbance of 1 at 260 nm	50 nM Primer
is .493 microMolar 4 and contains 26.9 micrograms.	50 mM Salt (Na^+)

Thermodynamic Constants
Conditions: 1 M NaCl at 25°C at pH 7.

RlogK 33.404 cal/(°K*mol)	deltaH 1409.4 Kcal/mol
deltaG 246.1 Kcal/mol	deltaS 3735.1 cal/(°K*mol)

To use this calculator, you must be using Netscape 3.0 or later or Internet Explorer version 3.0 or later, or another Javascript-capable browser
Self-Complementarity requires a 4.x browser. IE 5.0 is also supported.
This page was written in Javascript.
Extensively rewritten from 12/15/2000-12/19/2000 to isolate javascript Oligo object behaviors for teaching purposes.
This page may be freely distributed for any educational or non-commercial use.

About the Calculations

Therm dynamic Calculations

The nearest neighbor and thermodynamic calculations are done essentially as described by Breslauer *et al.*, *Proc. Nat. Acad. Sci.* **83**, 3746-50, 1986 (Abstract) but using the values published by Sugimoto *et al.*, *Nucl. Acids Res.* **24**, 4501-4505, 1996 (Abstract). This program assumes that the sequences are not symmetric and contain at least one G or C. The minimum length for the query sequence is 8.

The melting temperature calculations are based on the simple thermodynamic relationship between entropy, enthalpy, free energy and temperature, where

$$\Delta H = \Delta G + T\Delta S$$

The change in entropy (order or a measure of the randomness of the oligonucleotide) and enthalpy (heat released or absorbed by the oligonucleotide) are directly calculated by summing the values for nucleotide pairs obtained by Breslauer *et al.*, *Proc. Nat. Acad. Sci.* **83**, 3746-50, 1986. The relationship between the free energy and the concentration of reactants and products at equilibrium is given by

$$\Delta G = RT\ln\left(\frac{[DNA\bullet primer]}{[DNA][primer]}\right)$$

Substituting the two equations gives us

$$\Delta H = T\Delta S + RT\ln\left(\frac{[DNA\bullet primer]}{[DNA][primer]}\right)$$

and solving for temperature T gives

$$T = \frac{\Delta H}{\Delta S + R\ln\left(\frac{[DNA\bullet primer]}{[DNA][primer]}\right)}$$

We can assume that the concentration of DNA and the concentration of the DNA-primer complex are equal, so this simplifies the equation considerably. It has been determined empirically that there is a 5 (3.4 by Sugimoto et al.) kcal free energy change during the transition from single stranded to B-form DNA. This is presumably a helix initiation energy. Finally, adding an adjustment for salt gives the equation that the Oligo Calculator uses:

$$T = \frac{\Delta H - 5\dfrac{kcal}{°K\ mole}}{\Delta S + R\ln\left(\dfrac{1}{[primer]}\right)} + 16.6\log_{10}([Na^{+}])$$

No adjustment constant for salt concentration is needed, since the various parameters were determined at 1 Molar NaCl, and the log of 1 is zero.

ASSUMPTIONS:
The thermodynamic calculations assume that the annealing occurs at pH 7.0. The melting temperature (Tm) calculations assume the sequences are not symmetric and contain at least one G or C. The oligonucleotide sequence should be at least 8 bases long to give reasonable Tms.

Basic Melting Temperature (Tm) Calculations

The two standard approximation calculations are used. For sequences less than 14 nucleotides the formula is

Tm= (wA+xT) * 2 + (yG+zC) * 4

where w,x,y,z are the number of the bases A,T,G,C in the sequence, respectively.

For sequences longer than 13 nucleotides, the equation used is

Tm= 64.9 +41*(yG+zC-16.4)/(wA+xT+yG+zC)

ASSUMPTIONS:
Both equations assume that the annealing occurs under the standard conditions of 50 nM primer, 50 mM Na^{+}, and pH 7.0.

Salt Adjusted Melting Temperature (Tm) Calculati ns

A variation on two standard approximation calculations are used. For sequences less than 14 nucleotides the same formula as the basic calculation is use, with a salt concentration adjustment

$$Tm= (wA+xT)*2 + (yG+zC)*4 - 16.6*\log_{10}(0.050) + 16.6*\log_{10}([Na^{+}])$$

where w,x,y,z are the number of the bases A,T,G,C in the sequence, respectively.

The term **$16.6*\log_{10}([Na^{+}])$** adjusts the Tm for changes in the salt concentration, and the term **$\log_{10}(0.050)$** adjusts for the salt adjustment at 50 mM Na^{+}. Other monovalent and divalent salts will have an effect on the Tm of the oligonucleotide, but sodium ions are much more effective at forming salt bridges between DNA strands and therefore have the greatest effect in stabilizing double-stranded DNA.

For sequences longer than 13 nucleotides, the equation used is

$$Tm= 100.5 + (41 * (yG+zC)/(wA+xT+yG+zC)) - (820/(wA+xT+yG+zC)) + 16.6*\log_{10}([Na^{+}])$$

Symbols and salt adjustment term as above, with the term **(41 * (yG + zC-16.4)/(wA + xT + yG + zC))** adjusting for G/C content and the term **(820/(wA + xT + yG + zC))** adjusting for the length of the sequence.

ASSUMPTIONS:
Both equations assume that the annealing occurs under the standard conditions of 50 nM primer and pH 7.0.

OD Calculations

Molar Absorptivity values in 1/(Moles cm)

Residue	Moles^{-1} cm^{-1}	Molecular Weight (after protecting groups are removed)
Adenine (dAMP, Na salt)	15200	313.21
Guanine (dGMP, Na salt)	12010	329.21
Cytosine (dCMP, Na salt)	7050	289.18
Thymidine (dTMP, Na salt)	8400	304.2
6' FAM	20960	537.46
TET	16255	675.24
HEX	31580	744.13
TAMRA	31980	

Assume 1 OD of a standard 1ml solution, measured in a cuvette with a 1 cm pathlength.

6-FAM:

Chemical name:	**6-carboxyfluorescein**
Absorption wavelength maximum:	**495 nm**
Emission wavelength maximum:	**521 nm**
Molar Absorptivity at 260nm:	**20960** Moles^{-1} cm^{-1}

TET:

Chemical name:	**4, 7, 2', 7'-Tetrachloro-6-carboxyfluorescein**
Absorption wavelength maximum:	**519 nm**
Emission wavelength maximum:	**539 nm**
Molar Absorptivity at 260nm:	**16255** Moles^{-1} cm^{-1}

HEX:

Chemical name:	**4, 7, 2', 4', 5', 7'-Hexachloro-6-carboxyfluorescein**
Absorption wavelength maximum:	**537 nm**
Emission wavelength maximum:	**556 nm**
Molar Absorptivity at 260nm:	**31580** Moles^{-1} cm^{-1}

TAMRA:

Chemical name:	**N, N, N', N'-tetramethyl-6-carboxyrhodamine**
Absorption wavelength maximum:	**566 nm**
Emission wavelength maximum:	**580 nm**
Molar Absorptivity at 260nm:	**31980** $Moles^{-1}$ cm^{-1}

Nucleotide base codes (IUPAC)

Symbol: nucleotide(s)		
A adenine	**M** A or C	**K** G or t
C cytosine	**R** A or G	**V** A or C or G; not T
G guanine	**W** A or T	**H** A or C or T; not G
T thymine in DNA; uracil in RNA	**S** C or G	**D** A or G or T; not C
	Y C or T	**B** C or G or T; not A
N A or C or G or T		

Most recent version is available at URL: http://www.basic.northwestern.edu/biotools/oligocalc.html

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Oligo Calculator version 3.02 (last modified by WAKibbe 02/23/2002)